OCHRATOXIN A: TOXICOLOGICAL ASSESSMENT OF RESIDUES IN MEATS

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Ochratoxin A (OA) a dihydrocoumarin derivative linked to phenylalanin by an amino bond is a secondary metabolite produced by various species of the genera Penicillium and Aspergillus. As a result OA is found frequently in a number of cereal crops and related foods (for review see HARWIG et al., 1983). Under the German agricultural and environmental conditions OA is found predominantly in wheat and barley in concentrations ranging from 0.1-200 µg/kg, the mean concentration beeing 30 µg/kg feed (THALMANN and GRUBER, 1981; GEDEK, 1985; BAUER et al., 1986). The frequency of positive samples exceeds 10% for grains and formulated feeds and varies according to seasonal and regional factors. Cereals and crops for human consumption have, as of yet, not been monitored intensively. Recently JIAO et al. (1987) who investigated various oat, rey and barley meals as well as flours demonstrated that 50% of the products contained 0.1-0.3 µg/kg and 16.6% of the samples were contaminated with OA in concentrations ranging between 0.3 and 9.8 µg/kg.

After consumption of contaminated feedstuffs OA can be found in various edible tissues of pigs and poultry. Residues have been detected in blood serum, kidneys, liver, muscle and fat. Carcasses of ruminants (with the exception of preruminant veal calves) are unlikely to be contaminated as the microbial flora of the forestomach systeme degradates OA to less toxic metabolites capacitatively. Excretion of OA with eggs has been demonstrated in laying hens under experimental conditions (BAUER et al., 1988).

In Germany the occurence of OA has been monitored in plasma and kidneys of pigs. The residual concentrations in kidneys varied between 0.5 and 10.0 µg/kg, the majority of the samples ranging between 1-2 µg/kg tissue (BAUER et al., 1984; SCHEUER et al., 1984). Nearly 50% of the tested serum samples of pigs were contaminated with OA in concentrations between 0.1-67.3 µg/kg (BAUER et al., 1984). Consequently, sausages which were produced with pig plasma (frankfurter-type sausages) were contaminated as well, indicating the high stability of the toxin during processing of meats. In addition is it noticeable that the processing resulted not in a decrease of OA concentrations as the residual amount detected in sausages exceeded the mean concentrations in plasma samples two fold (SCHEUER, 1988).

Thus, OA has to be considered as a contaminant of foods of animal origin following the food chain as well as a contaminant of food of plant origin. However, it can not be estimated, as of yet, what are the food commodities which are predominantly responsible for human exposure. Human exposure can be assessed from two independent studies demonstrating that OA presently has to be expected in blood samples of more than 50% of all consumers (BAUER et al., 1986, SCHEUER and LEISTNER, 1986). In addition OA was found in some renal specimen derived from hospital patients (BAUER et al., 1986). Like in monogastric animals OA may be excreated with mothers milk resulting in an exposure of the neonatus (GAREIS et al., 1987). Therefore, it seems necessary to evaluate public health aspects of OA residues on the basis of available toxicological and kinetic data.

TARGET ORGAN TOXICITY

OA is a known nephrotoxin, inducing renal tubular necrosis, hepatic and lymphoid necrosis and a dessiminated intravascular coagulation like syndrome (KROGH, 1978; MEISNER and KROGH, 1986; ALBASSAM et al., 1987).

Subacute and subchronic toxicity studies in rats, guinea pigs, beagle dogs and pigs demonstrated that renal tubular necrosis can be observed even in low dosages. Subchronic administration

of OA (0.145 µg/kg b.w.) caused increased enzymuria and lower activities of tubular enzymes suggesting tubular injury (KANE et al., 1986). The dose dependent pathologic alterations can be estimated from a 90 day feeding experiment in rats, where 0.2 ppm OA (approximatively 0.01 mg/kg b.w.) resulted in minimal pathologic lesions in the kidneys and therefore could be accepted as the threshhold level of toxicity (MUNRO et al., 1974).

EMBRYOTOXICITY AND REPRODUCTIVE EFFECTS

OA is a potent teratogen in experimental dosages and causes malformations in mice, rats, hamsters and cockerels (HAYES, 1978). Additional studies demonstrated that the late implantation losses during pregnancy in rats were correlated with a suppression of ovarian steroidogenesis (GUPKA et al., 1981). In vitro experiments with short term cultured interstitial cells from gerbil testes gave evidence that testosterone synthesis might be affected as well (FENSKE and FINK-GREMMELS, 1989).

We conducted a set of experiments with the aim to establish a teratological no-effect-level. Reversible minor malformations (signs of delayed ossification of skeleton bones and wavy rips) could be observed at a mininum oral dose of 0.68 mg/kg b.w.. Fetal body weight was affected at a dose of 0.17 mg/kg b.w. when OA was applied to dams during the gestation period (THEIN et al., 1988). However, the most sensitive test parameter in these studies was a significant increase in placental weight, which could be observed at a dosage of 0.087 mg/kg b.w., indicating a maternal toxic effect of OA. Therefore, in risk assessment a teratological no-effect-level of 0.17 mg/kg b.w. corresponding to a feed concentration of 1.25 mg/kg can be accepted. These findings are comparable with previously published results (BROWN et al., 1976).

IMMUNOTOXICITY

The specific mode of action of OA is an inhibition of the phenylalanine-tRNA-synthetase, resulting in an inhibition of the elongation step in protein synthesis. Therefore, a reduced immunoglobuline synthesis has to be considered (CREPPY et al., 1984). This hypothesis is supported by pathohistological findings demonstrating necrotic lesions in various lymphoid tissues as well as by several in vitro tests (RICHARD et al., 1975, PRIOR and SISODIA, 1979; KLINKERT et al., 1981 and LUSTER et al., 1987).

Again, with the aim to establish a no-effect-level we performed a series of lymphocyte transformation tests, as an experimental model to assess altered cellular immune response under the influence of mycotoxins. This test can be developed with peripheral mononuclear cells from animals, which have been exposed in vivo to OA as well as for in vitro exposed cells. After oral administration to rats of 0.1, 0.5 and 1.0 mg OA/kg b.w. an inhibition of the proliferation rate of mitogen stimulated lymphocytes could be observed only in the highest dosage group (JAHN and FINK-GREMMELS, 1988). This dosage resulted in a mean cumulative serum concentration of 478 µg/l in the exposed rats. In addition, in vitro-tests with human derived peripheral blood lymphocytes indicated a mean effective concentration of 1108 µg/l cell suspension (v. GERNLER, JAHN and FINK-GREMMELS, 1989). When these data are compared to the OA concentrations which had been detected in human blood samples (0.1-0.9 µg/l) the broad safety margin between measurable effects in the lymphocyte transformation test and the natural occuring OA concentration is evident.

CARCINOGENICITY

Although previous studies failed to demonstrate a carcinogenic potential of OA in long term feeding experiments in rats (PURCHASE and van der WATT, 1971) and mutagenicity studies with bacterial test systems as well as genotoxicity tests in cell cultures were inconsistent (MORI et al., 1984; BENDELE et al., 1985a), recent animal experiments with mice and rats could clearly demonstrate an OA induced carcinogenesis (KANISAWA and SUZUKI, 1978; BENDELE et al., 1985b, BOORMAN, 1988).

TOXICOKINETICS

From several studies which have been conducted with different dosages and in various animal species it is evident, that absorption, distribution and elimination of OA are influenced by the route of administration and varies considerable among different animal species. Recently, HAGELBERG et al. (1989) compared the available data for OA. From this study it can be concluded that pigs and rats have related values for the plasma elimination rate (biological half-life of the toxin) with 150 and 170 hrs., respectively. However, in monkeys the OA half life is 510 hrs after oral administration of a similar dosage. The binding abilities of OA to plasma proteins were investigated as well, and in all species (including man) protein binding exceeded 99.8%. These kinetic investigations may help to interpret the data obtained from human blood sample surveys, as it remained so far unexplained why OA was found with a high incidence but in small, consistent amounts in man.

RISK ASSESSMENT

Mycotoxins are considered as environmental pollutants. An approach to assess the health risks to humans from the presence of OA residues in meats and other foods has to consider toxicological as well as kinetic data. In extrapolating to humans, the no-effect-level (NOEL) obtained from various animal studies together with safety factors have to be considered, the latter accounting interspecies and intraspecies differences as well as problems regarding from the suitability of the experimental models used. From subchronic experiments in rats (MUNRO et al., 1974) a NOEL of 0.01 mg/kg b.w. can be estimated for target organ toxicity. In extrapolation to humans the significance of the NOEL, a safety factor of at least 100 has to be applied, resulting in a tolerance level of 0.10 µg/kg b.w.. This exposure is comparable to the dosage which caused in subchronic feeding experiments in rats biochemically detectable, but reversible renal tubular effects (KANE et al., 1986).

For an assessment of the significance of embryotoxic and reproductive effects it is important that so far in all tested animal species the response to OA exposure during the gestation period resulted in dose dependent major or minor malformations. For the extrapolation of the significance of these animal experiments a safety factor of 500 should be used. Thus, for major malformations a tolerance level of 0.0014 mg/kg b.w. can be calculated. However, as a result of our studies and using the same safety factor an exposure of 0.17 µg/kg b.w. seems with a higher probability to indicate the tolerance level. Thus, both treshold levels for dose-dependent OA effects are in the same range and the safe intake estimated from these data is 0.10 µg/kg b.w./day.

The assessment of data on immunotoxicity is difficult. As mentioned above there seems to be a satisfying safety margin between the concentrations in human blood samples and the concentrations which cause measurable effects in in-vitro experiments with human derived lymphocytes.

However, experimental immunosuppressive effects are considered to be an indicator for carcinogenicity. The significance of tumorigenicity data in laboratory animals to man are discussed controversially. If a compound is avoidable and its usage is intended, as for example for hygienic or technical purposes in food production, carcinogens are considered as genotoxic, having no theshold level. Therefore, an estimation of an acceptable daily intake or a virtual safe dose is not accepted in food toxicology (FÜLGRAFF, 1989). Under this premise an environmental pollutant like a mycotoxin will be regulated with the aim to avoid any detectable residue - by means of the most sensitive method for food analysis.

RESIDUES IN CEREALS VS RESIDUES IN MEATS

From the limited survey data available on the natural occurence of OA in food commodities it can be estimated that cereals and other plant foods are contaminated with a higher incidence and in higher concentrations than foods of animal origin. However, for humans the absorption rate of OA from the different

sources has, as of yet, not been investigated. Therefore, it is impossible to discuss the relevance of protein bound residues in meat and meat products in comparison with OA exposure form plant materials. Additional studies on the bioavailability of OA from contaminated foods are necessary to elucidate the significance of the various food commodities in the OA food chain.

CONCLUSIONS AND RECOMMENDATIONS

Based on the toxicological date OA has to be considered an undesirable compound in foods for human consumption. Further studies are recommended to assess what food sources such as cereals or meats are predominantly responsible for human exposure. It is likely that for man the bioavailability for OA residues is higher in cereals than in meats, as in the latter OA is bounded to proteins.

Should future monitoring data indicate that pork contributes significantly to human OA exposure, guideline levels may need to be established. However, as feeds produced for on-farm usage are difficult to monitore, a screening method for the control of OA residues in pig carcasses should be developed.

From the toxicokinetic data it can be estimated that serum analysis will give sufficient information about OA contamination of carcasses. Further studies are necessary to define a plasma threshold level in pigs which does not result in any detectable residues in the carcass.

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